Pigment Spread in Guinea Pigs.

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It has been known for more than fifty years that the pigmentation of the superficial epidermis creeps from the colored into the white areas of recessively spotted guinea pigs, whether from grafts or, during normal life, across natural color boundaries. Analysis of the phenomenon has led to the suggestion that "pigment spread" is due to a permanent and serially propagable transformation of the dopa-negative, non-pigmentary dendritic cells of white skin areas into the pigmentary dendritic cells (epidermal melanophores or melanoblasts) characteristic of colored areas. According to this hypothesis, pigment spread is made possible, anatomically, by the fact that dendritic cells form a partially syncytial web throughout the basal layer of the epidermis, whatever its color pattern, and whether it is pigmented or not; and physiologically, by the fact that the epidermal dendritic cell is "cytocrine" in activity (Masson's term) and can presumably "infect" neighboring dendritic cells through a cytoplasmic anastomosis much as (in a trivial sense) it "infects" the Malpighian cells of the epidermis with melanin granules.

It is a shortcoming of this hypothesis that the non-pigmentary dendritic cells (leucophores or "white melanophores") of the colorless areas of spotted guinea pigs, on whose existence the truth of the hypothesis clearly depends, have not yet been seen in living skin. They are revealed by impregnation with acid gold chloride. This shortcoming is to some extent remedied by the fact that black pigmentation will encroach upon the *red* areas of spotted red-black or tricolor guinea pigs, though much more slowly than upon white. Red melanophores are visible in the living epidermis and are present in the same number and distribution as are black melanophores in black skin. Red skin transformed by pigment spread into black contains black melanophores only: either the red melanophores have been converted into black melanophores, or they have been by some unknown mechanism individually replaced and destroyed by them.

Epidermal epithelia lacking dendritic cells are non-infectable.

Pigmentation may be propagated from a black skin area of one guinea pig to a white skin area of another guinea pig if a minute quantity of a suspension in Ringer's solution of the basal layer cells of the black epidermis is "grafted" in such a way as to give the donor melanophores direct access to the "white" melanophores of the recipient. So initiated, foreign pigmentation has been observed to spread over an area as great as 500 mm^2, and in certain proportion of cases appears to survive indefinitely. But foreign pigmentation of any area or time of standing may be bleached out at will by transplanting skin from the donor animal whose cells started the foreign pigmentation to the recipient in which it is spreading. This response is thought to be the consequence of an active immunization, and it is believed that foreign pigmentation, though certainly *initiated* by living foreign cells, is maintained and propagated by the multiplication within host cells of some antigenically foreign cytoplasmic ingredient.

"Infective" behavior by constituents of the cytoplasm cannot but be a phenomenon of the greatest rarity: if the authors' interpretation is correct, it is made possible in the present instance by the cytocrine activity of dendritic cells, their semi-syncytial layout, and the co-existence of dendritic cells of different pigmentary activity in a single spotted guinea pig.

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Discussion by MORRIS FOSTER, Columbia University, New York

If the semi-syncytial network provides the path by means of which infectively transforming cytoplasmic particles pass from black-pigment-producing dendritic cells into "white" or into red-pigment-producing cells, and if the same type of transmission mechanism is responsible for "inoculating" Malpighian cells with melanin granules, then some infectively transforming particles should pass into the Malpighian cells along with the melanin granules, thereby infectively transforming the Malpighian cells as well. Since, however, Malpighian cells are not induced to form melanin, it is necessary to make subsidiary hypotheses. For example, one could assume that the cytoplasm in the Malpighian cell contains substances hostile to the action of the infectively transforming particles passing into it from the dendritic cell. If this be the case, however, then the cytoplasmic flow must always be in one direction only, into the Malpighian cell, since a reverse flow would inhibit pigment production in the dendritic cell.

Discussion by ERNST CASPARI, Wesleyan University, Middletown, Conn.

I was most interested to learn that the spreading phenomenon also occurs at the border of black and red areas, since this situtation might well provide critical evidence for the theory of transmission of pigment precursors. Under this theory it would be expected that cells containing both red and black granules would exist in the border region. I wonder particularly whether it would be possible to observe this phenomenon di-

rectly in tissue cultures of black and red melanophores.

Reply by Medawar.

The question of whether "infected" red melanophores contain both red and black pigmentary systems, or black alone, is certainly of first-rate importance. Although we are not very optimistic about the use of tissue culture, we hope that really detailed histological analysis will help to solve the problem.

Atypical Pigment Cell Differentiation in Embryonic Teleostean Grafts and Isolates.

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The occasional development of red blood corpuscles and of chromatophores is a condition which sometimes obtains in otherwise nondifferentiating isolates and grafts from young teleostean embryos (Oppenheimer, 1949). Examples of such atypical differentiation have occurred in three separate series of experiments (Oppenheimer, 1936a, 1938) to be discussed below.

Fundulus germ-ring grafts. In this series of experiments, portions of the germ-ring located 90° or 180° from the midline of the embryonic shield of *Fundulus heteroclitus* gastrulae (cf. fig. 1B, Oppenheimer, 1938) were grafted to the embryonic shield or to the extra-embryonic membrane of gastrulae of the same species. In 37 cases either the whole grafts or portions of them continued development but failed to undergo typical histogenesis and differentiated no axial structures. In 16 of these, however, red blood corpuscles differentiated, and in 10 of these 16 grafts melanophores differentiated. In some cases, only a single melanophore was differentiated within the grafts, in others a larger number was present. Only those grafts are included among the 10 positive cases which contained melanophores located more or less centrally in the mass of cells; grafts to which melanophores were applied only at the surface are excluded from consideration in the possibility that in these cases host melanophores might have migrated to cover the grafts.

Only heteroplastic transplantation or the grafting of materials from vitally stained embryos can furnish direct proof that the melanophores are formed by graft rather than host cells in such experiments as these. Fortunately, however, supplementary data are available from other types of experiment which demonstrate that chromatophores are also similarly differentiated in some cases by isolates rather than transplants from young teleostean embryos.

Epiplatys germ-ring isolates. The embryos of *Epiplatys fasciolatus* can be divided at late gastrula stages in such a way (cf. fig. 1A, Oppenheimer, 1938) that the portion of the germ-ring most remote from the embryonic shield can be isolated with part of the yolk from the portion of the egg containing the shield. Such isolates develop for several days in Ringer's solution. Though in some cases they exhibit certain phenomena of growth which simulate the form of the tail, they undergo no histogenesis of axial structures. Of 12 such aggregates studied, one developed red blood corpuscles, one formed a single melanophore and a third differentiated a number of xanthophores. One explant of prospective tailbud region, isolated immediately after the closure of the blastopore, formed both melanophores and xanthophores although otherwise undifferentiated.

Fundulus isolated blastoderms. Blastoderms of *Fundulus heteroclitus* separated from the yolk during cleavage stages and cultivated in double-strength Holtfreter's solution under some conditions form hyperblastulae, masses of nondifferentiated cells provided with a large vesicle at one pole (Oppenheimer, 1936a). Of approximately 75 hyperblastulae studied, only one developed a single melanophore of typical size and configuration.

Discussion and conclusions. The production of chromatophores by the grafts and isolates described above suggests that in the teleosts under certain experimental conditions pigment cells can be differentiated by cells which normally do not contribute to the teleostean counterpart of the neural crest. The 90° germ-ring of *Fundulus* normally contributes primarily mesoderm to the embryo (Oppenheimer, 1936b); and the 180° germ-ring is unrelated to nervous system formation except insofar as it contributes to the tailbud blastema from which neural derivatives may later arise. While it is theoretically possible, it is highly improbable that the chromatophores in these cases have been differentiated only by the particular cells destined to form them later after their passage through the tailbud blastema. The differentiation of pigment cells under these conditions is therefore presumably the result of what the experimental embryologists call *bedeutungsfremde Selbstdifferenzierung.*

This interpretation, however, is not incompatible with the possibility that in teleosts the chromatophores normally arise from cells corresponding to those of the neural crest of other vertebrates, as suggested by the results of the transplantation experiments of Lopashov (1944) on three species of teleosts, and in line with the neural crest